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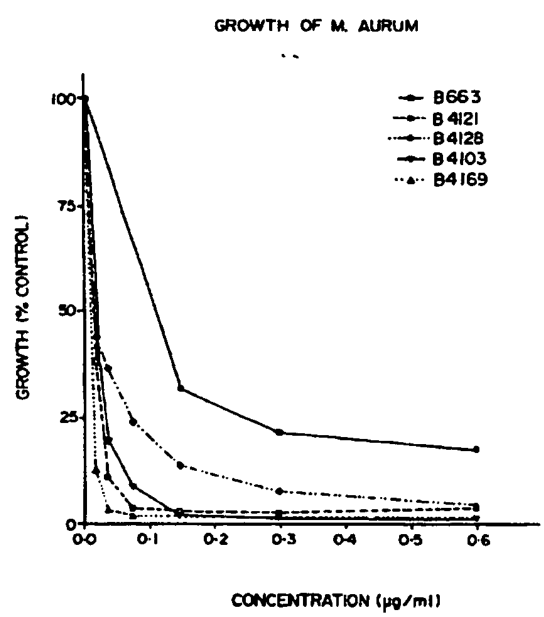
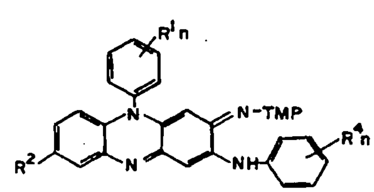
(54) Title: USE OF RIMINOPHENAZINES AS ANTIMICROBIAL AND ANTIMALARIAL AGENTS

(57) Abstract

A substance or composition for use in a method of treatment of a gram-positive bacterial infection of the human or animal body by the administration of said substance or composition to the human or animal body, said substance or composition comprising a compound of formula (II): in which R^1 and R^4 are the same and each is a halogen atom, a haloalkyl radical or an isopropyl radical, n is 1, 2 or 3 and represents the number of R^1 substituents and of R^4 substituents, R^2 is hydrogen, halogen or haloalkyl, and TMP is a tetramethyl piperidyl radical, with the provisos that (a) when n is 2, R^1n and R^4n are not 3,4-dichloro- and (b) when n is 1, R^1 and R^4 are not 4-trifluoromethyl-. The bacterial infection may be one or more of the species selected from *Mycobacterium aurum*, *Mycobacterium tuberculosis*, *Mycobacterium chelonae*, *Mycobacterium abscessus*, *Mycobacterium fortuitus*, *Streptococcuspneumoniae*, *Enterococcus* sp. and *Staphylococcus aureus*. The compounds may be administered in effective amounts. Also provided as novel pharmaceutically active compounds are N,5-*bis*(3-chloro-4-fluorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine and N,5-*bis*(2-chlorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine.





(II)

USE OF RIMINOPHENAZINES AS ANTIMICROBIAL AND ANTIMALARIAL AGENTS

THIS INVENTION relates to the use of compounds having antibacterial activity, to substances or compositions containing such compounds, to the manufacture of such substances or compositions and to methods of treatment using such compounds.

Recently bacterial strains resistant to every single chemotherapeutically useable antibacterial agent were identified among clinical isolates of some bacterial species. The rapid dissemination of multi-drug resistant bacteria from one hospital to another or from one country or even continent to another, linked to the tremendously increased mobility of human populations in the past decade, makes the public health hazard posed by these microbes a common environmental threat throughout our entire "global village".

In recent years, enterococci have become recognized as a common cause of nosocomial infections and were recently cited as being the second most common pathogen isolated from hospitalized patients. The increasing importance of enterococci in nosocomial infections may be partially related to the many inherent (i.e. natural occurring) and acquired resistances found in these organisms; these multiple resistances presumably provide a selective advantage allowing enterococci to survive many different antimicrobial regimens. Enterococcal microorganisms have decreased susceptibility to the majority of the ß-lactam antibiotics, aminoglycosides and, most significantly, vancomycin. Other gram-positive microorganisms, such as methicillin - resistant staphylococci also have decreased susceptibility to antibiotics. All of these resistances add to the clinical dilemma posed by enterococci because they decrease the number of available therapeutic options, and because they increase the number of agents whose use may provide a positive selective pressure for resistant organisms.

Microorganisms of the antibiotic-resistant species *Streptococcus pneumoniae* now have a global distribution. The brunt of the burden of resistance to

antimicrobial agents in pneumococci is experienced by children, because the resistance phenotype is predominantly distributed amongst serotypes prevalent in children. The risk factors for acquisition of these resistant strains include young age of the patient, hospitalization and exposure to antibiotics. A dramatic increase in the prevalence of penicillin-resistant pneumococci has been described, with 16% penicillin-resistant pneumococci reported in 1987 and 36% in 1992. Highly penicillin-resistant pneumococcal strains have been associated with treatment failures in both meningitis and otitis media. Because of the poor penetration of ß-lactam antibiotics (e.g. penicillin) into the cerebro-spinal fluid (hereinafter referred to as CSF), pneumococcal meningitis caused by penicillin-resistant strains will frequently not respond adequately to penicillin therapy. The extended-spectrum cephalosporins, cefotaxime and ceftriaxone, have therefore become regarded as the drugs of choice for penicillin-resistant pneumococcal meningitis. Unfortunately, a case of cefotaxime treatment failure of an 8 month old baby with pneumococcal meningitis has already been reported. It is therefore evident that existing therapy of pneumococcal meningitis is not fool-proof and treatment failures with existing agents necessitate the development of alternative therapeutic agents and strategies.

Rifampicin is a potent first-line anti-tuberculosis drug which possesses impressive activity against human macrophages. However, there is world-wide concern about the problem of multi-drug resistant tuberculosis and an urgent need for new anti-tuberculosis drugs.

It would be desirable to provide a new group of compounds which have antibacterial activity and to which microorganisms are not resistant.

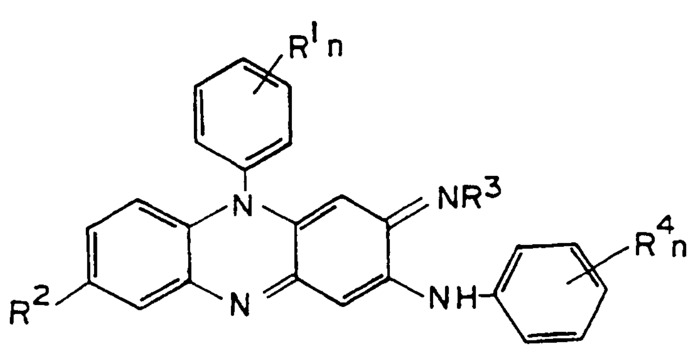
South African Patent No. 95/1840 and corresponding applications in other countries, (such as European Patent Application No. 95301772.0) describe and claim a group of riminophenazines which have been found to have activity as MDR agents, i.e. activity against multi-drug resistance, particularly for use in the treatment of cancer patients. The riminophenazines are phenazines which contain

a substituent on a ring nitrogen atom and a substituted or unsubstituted imino substituent in one of the benzene rings. The imino group conveniently may be in the 2- or 3- position, the nitrogen atoms of the phenazine being in the 5- and 10- positions. Conveniently, there may also be an amino group in the same benzene ring as the imino group, preferably in the 3- or 2- position. The riminophenazines generally had a 2-(substituted amino)-3-(substituted imino)-5-aryl grouping optionally with a further substituent in the 8- position.

The novel riminophenazines described in the aforesaid EPO application 95301772.0 have also been found to have anti-parasitic activity, eg. against the malarial parasite, *Plasmodium falciparum*. The use of some of the riminophenazines in the treatment of anti-parasitic diseases was described and claimed in European patent application EPO 729 757, and corresponding applications in other countries.

Some of the riminophenazines which showed MDR and anti-malarial activity were known compounds which had other activities. For example, activity against microorganisms of genus *Mycobacterium* has been reported, eg. in Antimicrobial Agents and Chemotherapy, March 1996, pages 633-636; Biochemical Society Transactions (1995), vol 23, page 357S; International Journal of Leprosy, vol 61, September 1993, pages 406 - 414 and Health Cooperation Papers N12, 1992, pages 191 - 197.

A preferred group of phenazines of the aforesaid European patent applications were compounds of the general formula (I), i.e.



(I)

in which R^1 is a hydrogen atom, a halogen atom, or an alkyl, alkoxy or fluoroalkyl radical,

R_2 is a hydrogen or halogen atom,

R^3 is selected from hydrogen, alkyl, substituted alkyl, cycloalkyl, cycloalkylalkyl or is a substituted or unsubstituted heterocyclic or heterocyclic-alkyl radical,

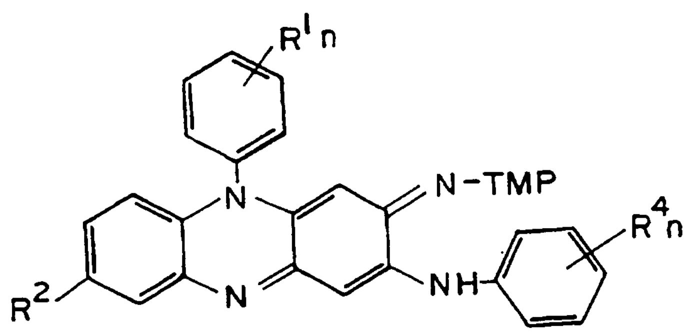
R^4 is a hydrogen or halogen atom or an alkyl, alkoxy or fluoroalkyl radical, and

n is 1, 2 or 3.

Among those compounds were a number of compounds in which the heterocyclic radical R^3 was a substituted heterocyclic radical, particularly a TMP radical (i.e. a 2,2,6,6-tetramethyl piperidyl radical) attached to the nitrogen atom in the 4- position of the heterocyclic ring.

We have now surprisingly found that a limited group of riminophenazines which have a 4-TMP radical attached to the imino nitrogen atom have shown particularly good antibacterial activity against gram-positive microorganisms, especially against bacteria of the genera *Mycobacterium, Enterococcus, Streptococcus* and methicillin-resistant *Staphylococcus*.

The compounds which show this good antibacterial activity are those of the general formula (II):



(II)

in which R^1 and R^4 are the same and each is a halogen atom, a haloalkyl radical or an isopropyl radical, n is 1, 2 or 3 and represents the number of R^1 substituents and of R^4 substituents, R^2 is hydrogen, halogen or haloalkyl, and TMP is a tetramethyl piperidyl radical with the proviso that when n is 2, R^1n and R^4n are not each 3,4-dichloro.

R^1 and R^4 are the same as one another but where n is 2 or 3, they may represent different substituents for different positions on the phenyl groups.

The compounds of formula (II) can be made using known procedures described for the preparation of riminophenazines, e.g. the procedures specified in South African Patent Application No. 95/1840 and its corresponding applications in other countries.

Presently preferred compounds of general formula II are those in which R^1n and R^4n each represent the following substituents at the positions given in the benzene rings in which they are substituents:

4-trifluoromethyl-
2-chloro-
3-chloro-
3-chloro-4-fluoro-
3,5-dichloro-
3-trifluoromethyl-
2,4-dichloro-
3-trifluoromethyl-4-chloro-
3,4,5-trichloro-
3-bromo-
3-fluoro-
4-isopropyl-

R^2 is hydrogen or chlorine, preferably hydrogen, and the TMP radical is a 2,2,6,6-tetramethyl piperidyl radical attached to the imino nitrogen atom at the 4-position of the piperidyl ring.

The compounds of the above general formula (II) specified above, are active against, for example, *Mycobacterium aurum, Mycobacterium tuberculosis, Mycobacterium chelonae, Mycobacterium abscessus, Mycobacterium fortuitus, Streptococcus pneumoniae, Enterococcus sp.* and *Staphylococcus aureus.*

In a first aspect, therefore, the invention provides a substance or composition for use in a method of treatment of gram-positive bacterial infection of the human or animal body by the administration of said substance or composition to the human or animal body, said substance or composition comprising a compound of formula (II) in which R^1, R^2, R^4, n and TMP have the meanings defined above for formula (II) with the provisos that (a) when n is 2, R^1n and R^4n are not 3,4-dichloro- and (b) when the gram-positive infection is caused by the genus *Mycobacterium* and n is 1, R^1 and R^4 are not 4-trifluoromethyl-.

In a second aspect, the invention provides the use, in the manufacture of a substance or composition for administration to persons or animals suffering from bacterial infection caused by gram-positive bacteria to treat the infection, of a compound of the formula (II) above in which R^1, R^2, R^4, n and TMP have the meanings defined above for formula (II) with the provisos that (a) when n is 2, R^1n and R^4n are not 3,4-dichloro- and (b) when the gram-positive infection is caused by the genus *Mycobacterium* and n is 1, R^1 and R^4 are not 4-trifluoromethyl-.

In a third aspect, the invention provides a method of treating a human or animal patient suffering from bacterial infection by gram-positive bacteria, which comprises administering to the human or animal patient an effective amount of a compound of the formula (II) above in which R^1, R^2, R^4, n and TMP have the meanings defined above for formula (II) with the proviso that (a) when n is 2, R^1n

and R^4n are not 3,4-dichloro- and (b) when the gram-positive infection is caused by the genus *Mycobacterium* and n is 1, R^1 and R^4 are not 4-trifluoromethyl-.

Compositions containing the compounds of formula (II) can be made up in the normal manner for antibacterial compositions, e.g. as tablets, sterile solutions, capsules or the like. Since many bacteria have become resistant to treatment with antibiotics, it is desirable to introduce the compounds of formula (II) to the bloodstream rapidly, e.g. by injection or infusion. The invention particularly provides liquid compositions for injection or infusion comprising a compound of formula (II) and a sterile liquid carrier.

Examples of compounds of formula (II), which have been found to be particularly suitable as antibacterials are set out in the following Table A. TMP is the radical 2,2,6,6-tetramethylpiperidyl in each compound.

TABLE - A

Compound	R^1n	R^2	R^4n
B4103	4-CF_3-	H	4-CF_3-
B4112	3-Cl-	H	3-Cl-
B4119	3-Cl-4-F-	H	3-Cl-4-F-
B4121	3,5-di-Cl-	H	3,5-di-Cl-
B4125	2-Cl-	H	2-Cl-
B4126	3-CF_3-	H	3-CF_3-
B4127	3-CF_3-	Cl	3-CF_3-
B4128	2,4-di-Cl-	H	2,4-di-Cl-
B4158	4-isopropyl-	H	4-isopropyl-
B4163	3-CF_3-4-Cl-	H	3-CF_3-4-Cl-
B4169	3,4,5-tri-Cl-	H	3,4,5-tri-Cl-
B4180	3-Br-	H	3-Br-
B4322	3-F-	H	3-F-

It is to be understood that the new activity for B4103 is against infections caused by the genera *Streptococcus*, *Enterococcus* and *Staphylococcus*.

The activity of the different compounds of formula (II) varies depending on the specific gram-positive bacteria being tested. For example, compounds B4121, B4126 and B4169 appear to be very active against bacteria of the genus *Mycobacterium*, whereas good activity was found for all of the aforesaid compounds against microorganisms of the genus *Streptococcus*, with compounds B4103, B4119, B4121 and B4127 showing the best activity of the compounds of formula II against many different isolates. The compounds B4126 and B4127 appear to show good activity against microorganisms of the genus *Enterococcus*.

B4169 and B4125, and to a lesser extent B4128 showed good activity against *M. tuberculosis* in a comparative test. General interest is presently centred on B4119, B4121, B4125 and B4126. The riminophenazines may be administered in non-toxic amounts but sufficient to combat the infection and/or to maintain protection against the infection caused by the gram-positive bacteria concerned. The amounts vary from compound to compound and can be determined by experimentation.

Compounds B4119 and B4125 of Table A are novel and thus, in fourth and fifth aspects, the invention provides the following novel pharmaceutically active compounds for use in the treatment of the human or animal body, said compounds being

N,5-*bis*(3-chloro-4-fluorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine; and

N,5-*bis*(2-chlorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine.

This pair of riminophenazines show activity as potential therapeutic agents, eg. multi-drug resistance compounds in the treatment of cancer (i.e. MDR activity) and very good activity as anti-parasitic agents, particularly as anti-malarial agents.

As anti-malarial agents, B4119 and B4125 show considerably high activity at relatively low concentrations against *Plasmodium falciparum*. Thus, B4119 and B4125 can be used in the manufacture of medicaments to treat infections caused by parasites, e.g. malaria transmitted by mosquitoes. Either of the compounds may be used as the sole active ingredient, or may be used together with other compounds which have anti-parasitic activity. The treatment may be by means of a single composition containing either of the compounds and another anti-parasitic compound, or by separate compositions, one containing either of the compounds and the other containing another anti-parasitic compound.

The invention further provides a method for the prophylactic and/or therapeutic treatment of parasitic infection of the human or animal body, which comprises administering an effective amount of B4119 or B4125 to the human or animal body.

The invention further provides a substance or composition for use in the treatment of infection caused by parasites, said substance or composition containing B4119 or B4125.

The invention further provides novel pharmaceutical compositions containing the compounds B4119 and B4125, eg. in unit dosage form.

The invention also provides a method for the preparation of B4119 or B4125, which comprises preparing these compounds using methods known *per se* for the preparation of riminophenazines. For example, the processes described in South African Patent No. 95/1840 may be followed. That Patent claims riminophenazines *per se*, their preparation and the use of riminophenazines in the treatment of multi-drug resistance in cancer patients.

For example, a 1-anilino-2-nitrobenzene, appropriately substituted in the anilino phenyl ring (i.e. by 3-Cl-4-F- or by 2-Cl-) may be reduced with hydrogen in the presence of a palladium carbon catalyst, or in zinc and acetic acid to form the

corresponding 1-anilino-2-aminobenzene (i.e. a 2-aminodiphenylamine containing the appropriate substituents), e.g. by heating at 40 to 55 °C. This diphenylamine may be oxidatively condensed, e.g. with ferric chloride and concentrated hydrochloric or acetic acid to form the riminophenazine corresponding to B4119 or B4125 but in which the imino group ($=NR^3$) in the 3-position is unsubstituted (i.e. R^3 is hydrogen). The use of ambient temperatures of preferably below 15 °C may be used and the reaction carried out in ethyl alcohol. This riminophenazine can be reacted with 4-amino-TMP and refluxed in dioxane for three to five hours to form B4119 or B4125.

Compositions containing B4119 or B4125 can be made up in the normal manner for pharmaceutical compositions. Any suitable appropriate carrier or solvent may be used. The active ingredient, namely the riminophenazine, may be present in any suitable form, eg. as the free base or as a suitable pharmaceutically acceptable salt thereof.

In addition to antimicrobial activity against antibiotic resistant *Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus* sp. and a vancomycin resistant *Enterococcus faecium* isolate and very good anti-malarial activity, B4119 and B4125 also show anti-tumour activity and MDR activity. B4125 also shows direct anti-mycobacterial activity as well intracellular anti-mycobacterial activity against *Mycobacterium tuberculosis*.

The compounds B4119 and B4125 have potential in the treatment of Multi-Drug Resistant tumours. Multi-Drug Resistance can either be acquired by tumours during the course of chemotherapy or it can be an intrinsic characteristic. Examples of tumours in the former class, where MDR emerges in the course of treatment, are those of acute leukemias, breast cancer and lymphomas. On the other hand, the intrinsic tumours of, for example, colon cancer, liver cancer, oesophageal cancer and prostate cancer, exhibit an initial low response rate to chemotherapy.

Intrinsic MDR tumours may have several coexistent MDR mechanisms and appear to be largely refractory to the usual MDR modulation strategies. The identification of agents to circumvent intrinsic MDR represents a major challenge to those involved with the development of anti-cancer agents. The compounds B4119 and B4125 address that challenge.

In three final aspects, the invention provides, respectively, for the compound B4103;

- A substance or composition for use in a method of treatment of bacterial infection of the human or animal body caused by bacteria of the genus *Streptococcus, Enterococcus* or *Staphyloccus*, said method of treatment comprising administering an effective amount of said substance or composition to the human or animal body, said substance or composition comprising N,5-*bis*(4-trifluoromethylphenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

- The use, in the manufacture of a substance or composition for administration to persons or animals suffering from bacterial infection by bacteria of the genus *Streptococcus, Enterococcus* or *Staphylococcus* to treat the infection, of the compound N,5-*bis*(4-trifluoromethylphenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine; and

- A method of treating a human or animal patient suffering from bacterial infection by bacteria of the genus *Streptococcus, Enterococcus* or *Staphylococcus*, which comprises administering to the human or animal patient an effective amount of N,5-*bis*(4-trifluoromethylphenyl)-3,5-dihydro-3- [(2', 2', 6', 6' -tetramethyl-4-piperidyl)-imino]-2- phenazinamine.

The invention is illustrated in non-limiting manner by reference to the following Examples, the results of which are shown in the tables and accompanying drawings, as appropriate.

In the accompanying drawings:

Figure 1 shows the results of the growth of a *M. aurum* strain against the concentration of riminophenazine, according to the experiment described in Example 1;

Figure 2 shows the growth of a *M. tuberculosis* strain H37Ra against the concentration of riminophenazine, according to the experiment described in Example 1;

Figure 3 shows the growth of the *M. tuberculosis* strain H37Rv against the concentration of riminophenazine according to the experiment described in Example 2;

Figure 4 shows the intracellular survival of *M. tuberculosis* H37Rv against the concentrations of riminophenazine according to the experiment described in Example 5; and

Figure 5 shows the anti-malarial activity of B4119 and B4125, as well as two known compounds, against *P. falciparum*.

EXAMPLE 1

With a view to finding an effective anti-tuberculosis agent, various riminophenazines were tested using *in vitro* screening procedures. Clofazimine (B663) was used as a standard.

Mycobacterial strains

Two *Mycobacterium tuberculosis* strains (H37Rv ATCC 27294) and its mutant (H37Ra ATCC 25177) were obtained from the Medical Research Council, Pretoria, South Africa. A *Mycobacterium aurum* strain obtained from the Pasteur Institute was also included. It has been described in Antimicrobial Agents and Chemotherapy 1995, 39(10); 2235-8.

Methods

The riminophenazines were dissolved in 100 % ethanol to give stock concentrations of 2 mg/ml. Subsequent dilutions were made in ethanol.

Suspensions of the organisms were made up to match a McFarland no. 1. The riminophenazines were dispensed into vials containing BACTEC 12B medium to give the required concentrations. These vials were then inoculated with 0.1 ml of a 1/10 dilution of the suspension in the case of *M.aurum* and 1/20 in the case of *M.tuberculosis H37Ra.* Solvent controls were included throughout. Vials were incubated at 37 °C for 48 hours and bacterial growth recorded on a BACTEC 460 reader.

Results

Results are expressed as a percentage of the solvent control. The results obtained with *M.aurum* are shown in Figure 1, and the results with *M.tuberculosis* H37Ra are shown in Figure 2 of the accompanying drawings. The agents B4169 and B4121 were the most active compounds of formula (II) against the Mycobacterial strains tested.

EXAMPLE 2

M.tuberculosis H37Rv (ATCC 27294) was grown in BACTEC 12B medium vials until the growth index (GI) reached 500 as measured by a BACTEC 460 reader.

The riminophenazines were dispensed into vials containing BACTEC 12B medium to give the required concentrations. These vials were then inoculated with 0.1 ml of the culture. Solvent controls were included. Vials were incubated at 37°C for 3 days and read on a BACTEC 460 reader. The riminophenazines tested

were B4121, B4125, B4128, B4158 and B4169, with B663 also being tested as a standard.

Results

Results are expressed as percentage of the solvent control. The results obtained with *M.tuberculosis* treated with B663, B4121, B4128, B4125, B4158 and B4169 are shown in Figure 3 of the accompanying drawings.

Conclusions

Of all of the agents tested, B4169, B4121 and B4125 are the three most active agents against *M.tuberculosis* H37Rv.

EXAMPLE 3

B4121, with clofazimine (B663) as reference standard, were dissolved and mixed with 7h10 agar medium at the relevant concentrations in plates. These plates were inoculated with different strains of Mycobacteria and incubated for differing time periods. Both rapid-growing and slow-growing bacteria were used in this Example.

Results

a. Rapid-growing organism.

Plates were read after 2 days. [MIC denotes Minimum Inhibitory Concentration.]

Culture	MICs (µg/ml) B4121	B663 (clofazimine)
M.chelonae (96012*)	1.25	2.5
M.abscessus (23259*)	1.25	2.5
M.abscessus (33777*)	1.25	5.0
M.fortuitus (44806*)	1.25	2.5

* These are laboratory strains obtainable from the Medical Research Council of South Africa.

b. *Mycobacterium tuberculosis* (slow-growing organisms). Plates were read after 2 weeks. In the following table:

INH means isoniazid hydrochloride
RMP means rifamyim
SM means streptomycin
EMP means ethambutol

Apart from H37Rv, all strains were laboratory strains obtainable from the Medical Research Council of South Africa.

Culture	Resistance	MICs (µg/ml) B663(clofazimine)	B4121
171 lab strain	INH, RMP	0.63	0.31 - 0.63
178 lab strain	INH, RMP, SM	0.63	0.31
218 lab strain	INH	0.63 - 1.25	0.31
H37Rv(ATCC 27294)	Susceptible	0.63	0.31 - 0.63
4591 (Refilwe - lab strain)	RMP	0.63	0.31 - 0.63
46 lab strain	INH,RMP,SM,EMB	0.63	0.31 - 0.63

The laboratory strains are obtainable from the University of Pretoria.

The results show that B4121 is clearly a more active compound than B663 against all the *Mycobacterium* strains tested.

EXAMPLE 4

The *in vitro* activity of compounds of general formula (II) were examined against multiple-drug resistant *Enterococcus* sp. and *Streptococcus pneumoniae* and the methicillin-resistant *Staphylococcus aureus* strains described in Table 3 below.

Method and bacterial strains

Clofazimine (as a standard) and the riminophenazines of formula (II) given in Table A above were included in experiments with *S.pneumoniae*, *S. aureus* and *Enterococcus* sp. Stock solutions of 2 mg/ml were used throughout. Subsequent dilutions were made in sterile, distilled water.

The bacterial strains that were used in this study, their origins and drug resistance profiles are listed in Tables 1 and 2 below. Quality control organisms that were included in this study are as follows: *Streptococcus pneumoniae* ATCC 6305 *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213.

In vitro susceptibility tests:

Agar dilution drug susceptibility tests were carried out on all strains according to standardized methods described by the National Committee for Clinical Laboratory Standards (NCCLS) document no. M7-A2. Included in these experiments was a test to establish whether exposure to sub-inhibitory drug concentrations could lead to the development of resistance to these drugs.

<u>Results</u>

The results (minimum inhibitory concentrations, or MIC values) of the activity of the riminophenazines against multiple-drug resistant *Streptococcus pneumoniae* strains are shown in Table 4 below. The effects of the riminophenazines against multiple-drug resistant *Enterococcus* sp. are shown in Table 5 below. The MIC values in the tables are given in μg/ml. Table 6 below shows results of the activities of the riminophenazines against methicillin-resistant *S.aureus* strains.

Compounds B4103, B4119, B4121, B4125 and B4126 showed good results against *Streptococcus pneumoniae*, while B4125 and B4126 were the most active against *Enterococcus* sp. The most active riminophenazines against the methicillin-resistant *S.aureus* strains are B4119, B4125 and B4126.

CSF in Table 1 is the abbreviation for cerebro-spinal fluid.

Table 1: Descriptions of the *Streptococcus pneumoniae* strains used in this study.

Strain no.	Source	Resistance to antimicrobial agents
1	Blood	Penicillin
2	Blood	Penicillin, Cefotaxime, Tetracycline, Erythromycin, Clindamycin
3	Blood	Penicillin
4	Control	None
5	CSF	Penicillin, Cefotaxime
6	Blood	Penicillin, Erythromycin, Tetracycline, Clindamycin
7	Blood	Penicillin
8	Blood	Rifampicin
9	CSF	Rifampicin
10	CSF	Rifampicin

Table 2: Descriptions of the *Enterococcus* sp. used in this study.

Strain no.	Species	Source	High-level resistance to antimicrobial agents
1	*faecalis*	Blood	Penicillin, Ampicillin and Gentamicin
2	*faecalis*	Blood	"
3	*faecium*	Blood	"
4	*faecium*	Blood	"
5	*faecium*	Blood	"
6	*faecalis*	Blood	"
7	*faecalis*	Blood	"
8	*faecalis*	Blood	"
9	*faecalis*	Blood	"
10	*faecalis*	Blood	"
11	*faecium*	Blood	"
12	*faecalis*	Blood	"
13	*faecium*	Blood	"
14	*faecium*	Blood	"
15	*faecium*	Blood	"
16	*faecium*	Blood	"
17	*faecium*	Blood	"
18	*faecium*	Blood	"
19	*faecalis*	Blood	"
20	*faecium*	Blood	"
21	*faecium*	Blood	"
22	*faecium*	Blood	"
23	*faecium*	Urine	"
24	*faecium*	Aspirate	"

Table 2: ..../continued

Strain no.	Species	Source	High-level resistance to antimicrobial agents
25	*faecium*	Blood	"
26	*faecium*	Pus	"
27	*faecium*	Urine	"
28	*faecium*	Blood	"
29	*faecium*	Blood	"
30	*faecium*	Blood	"
31	*faecium*	Blood	"
32	*faecium*	Pus	"
33	*faecium*	Pus	"
34	*faecalis*	Pus	"
35	*faecium*	Blood	"
36	*faecium*	Blood	"

Table 3: Descriptions of the methicillin-resistant *Staphylococcus aureus* strains used in this study.

Strain no.	Source
1	Pus swab
2	Pus swab
3	Pus swab
4	Blood
5	Blood
6	Blood
7	Blood
8	Blood
9	Blood
10	Blood
11	Blood
12	Pus
13	Pus swab
14	Central venous point
15	Pus swab
16	Pus swab
17	Pus swab
18	Pus swab
19	Central venous point
20	Pus
21	Pus swab
22	Pus
23	Pus
24	Abdominal fluid

Table 4: The activity of the riminophenazines against multiple drug resistant *Streptococcus pneumoniae* isolates

	Minimum Inhibitory Concentrations (MICs) of clofazimine and its derivatives										
Isolate no.	B663 (μg/ml)	B4103 (μg/ml)	B4112 (μg/ml)	B4119 (μg/ml)	B4121 (μg/ml)	B4126 (μg/ml)	B4127 (μg/ml)	B4128 (μg/ml)	B4163 (μg/ml)	B4169 (μg/ml)	B4180 (μg/ml)	B4322 (μg/ml)
1	1	2	2	2	1	>2	1	2	2	>2	>2	>2
2	<0.125	2	1	<0.125	1	<0.125	<0.125	0.5	0.25	1	>2	>2
3	1	<0.125	>2	<0.125	<0.125	1	1	2	2	1	<0.125	<0.125
4	1	0.25	>2	>2	1	2	1	2	2	>2	>2	2
5	1	1	>2	2	1	2	1	2	2	>2	>2	>2
6	0.25	2	1	2	1	0.25	0.25	1	0.25	>2	>2	>2
7	0.25	<0.125	<0.125	1	<0.125	<0.125	<0.125	<0.125	<0.125	>2	<0.125	<0.125
8	2	<0.125	2	0.5	<0.125	2	1	1	2	2	<0.125	<0.125
9	1	<0.125	2	0.5	0.5	2	1	2	2	2	>2	2
10	1	<0.125	>2	<0.125	<0.125	2	1	2	2	>2	<0.125	>2
ATCC*	1	0.5	1	2	2	1	1	1	2	1	2	2

* ATCC is the control strain *Enterococcus pneumoniae* ATCC 49169

Table 5: Activity of the riminophenazines against multiple drug resistant *Enterococcus* sp.

	Minimum Inhibitory Concentrations (MICs) of clofazimine and its derivatives											
Isolate no.	B663 (µg/ml)	B4103 (µg/ml)	B4112 (µg/ml)	B4119 (µg/ml)	B4121 (µg/ml)	B4126 (µg/ml)	B4127 (µg/ml)	B4128 (µg/ml)	B4163 (µg/ml)	B4169 (µg/ml)	B4180 (µg/ml)	B4322 (µg/ml)
1	>2	2	2	>2	>2	2	2	2	2	>2	>2	>2
2	>2	2	2	>2	>2	2	2	2	2	>2	2	>2
3	>2	>2	>2	>2	>2	1	1	2	2	>2	>2	>2
4	>2	>2	>2	2	>2	2	>2	2	>2	>2	>2	>2
5	>2	>2	>2	>2	>2	2	>2	2	>2	>2	>2	>2
6	>2	2	2	>2	>2	2	>2	2	>2	>2	>2	>2
7	>2	2	2	2	>2	2	2	2	1	>2	>2	>2
8	>2	2	2	>2	>2	2	2	2	2	>2	>2	>2
9	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
10	>2	2	2	>2	>2	1	>2	>2	>2	>2	>2	>2
11	>2	2	>2	>2	2	1	>2	>2	>2	>2	2	>2
12	>2	1	2	>2	1	2	1	>2	2	>2	2	>2
13	>2	>2	2	>2	>2	2	>2	2	2	>2	>2	>2
14	>2	>2	2	>2	>2	1	2	2	2	>2	>2	>2
15	>2	2	2	>2	2	1	1	2	2	>2	>2	>2
16	>2	2	2	>2	2	1	2	2	2	>2	>2	>2
17	>2	>2	>2	>2	>2	2	>2	2	>2	>2	>2	>2
18	>2	>2	>2	>2	>2	2	>2	>2	>2	>2	>2	>2

Table 5: continued

Minimum Inhibitory Concentrations (MICs) of clofazimine and its derivatives											continued
Isolate no.	B663 (μg/ml)	B4103 (μg/ml)	B4112 (μg/ml)	B4119 (μg/ml)	B4121 (μg/ml)	B4126 (μg/ml)	B4127 (μg/ml)	B4128 (μg/ml)	B4163 (μg/ml)	B4169 (μg/ml)	B4180 (μg/ml)	B4322 (μg/ml)
19	>2	2	2	>2	>2	2	>2	>2	>2	>2	>2	>2
20	>2	2	>2	>2	>2	2	>2	2	>2	>2	>2	>2
21	>2	2	>2	2	>2	1	2	2	>2	>2	>2	>2
22	>2	2	>2	>2	>2	2	>2	2	>2	>2	>2	>2
23	>2	2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
24	>2	>2	>2	>2	>2	2	>2	2	>2	>2	>2	>2
25	>2	2	2	>2	>2	1	2	2	2	>2	2	>2
26	>2	>2	>2	>2	>2	2	2	2	>2	>2	>2	>2
27	>2	1	>2	>2	2	2	>2	2	>2	>2	>2	>2
28	>2	>2	>2	>2	>2	2	>2	2	>2	>2	>2	>2
29	>2	2	2	>2	>2	1	>2	2	>2	>2	2	>2
30	>2	2	>2	>2	2	1	>2	>2	>2	>2	2	>2
31	>2	2	>2	>2	2	2	>2	>2	>2	>2	2	>2
32	>2	2	>2	>2	>2	1	2	2	2	>2	2	>2
33	>2	2	>2	>2	2	2	2	2	>2	>2	>2	>2
34	>2	2	>2	>2	>2	2	2	>2	>2	>2	>2	>2
35	>2	2	>2	>2	2	2	2	2	>2	>2	>2	>2
36	>2	2	>2	>2	2	1	1	2	2	>2	>2	>2
ATCC*	>2	2	2	>2	>2	2	>2	>2	>2	>2	2	>2

* ATCC is the control strain *Enterococcus faecalis* ATCC 29212.

Table 6: Activity of Clofazimine and its derivatives against isolates of *Staphylococcus aureus*

Isolate no.	B663 (µg/ml)	B4103 (µg/ml)	B4112 (µg/ml)	B4119 (µg/ml)	B4125 (µg/ml)	B4126 (µg/ml)	B4127 (µg/ml)	B4128 (µg/ml)	B4158 (µg/ml)	B4163 (µg/ml)	B4169 (µg/ml)	B4180 (µg/ml)	B4322 (µg/ml)
ATCC	>2	1	2	1	1	0.25	>2	>2	1	0.25	0.5	2	2
2	>2	1	2	0.5	>2	>2	>2	>2	>2	1	>2	2	2
2	>2	1	1	1	<0.125	0.25	>2	>2	1	0.25	<0.125	1	2
3	>2	1	2	1	>2	>2	>2	>2	>2	0.5	>2	2	2
4	>2	1	2	0.5	>2	>2	2	>2	>2	1	>2	2	1
5	>2	1	1	2	>2	>2	>2	>2	>2	1	2	1	1
6	>2	1	1	2	<0.125	0.25	>2	>2	1	0.25	0.5	1	1
7	>2	<0.125	1	1	1	>2	1	2	1	0.25	0.25	0.5	2
8	>2	0.25	1	2	1	>2	1	2	2	1	0.25	0.5	2
9	>2	1	1	2	>2	>2	>2	>2	>2	1	0.25	2	2
10	>2	1	2	1	<0.125	>2	2	2	1	0.25	0.25	1	2
11	>2	0.5	0.5	1	<0.125	>2	2	2	1	0.25	<0.125	1	2
12	>2	0.5	1	0.5	>2	>2	2	2	>2	1	>2	1	1
13	>2	0.5	0.5	0.5	>2	0.125	1	2	1	0.25	<0.125	0.5	1
14	>2	0.25	0.5	0.5	<0.125	0.125	1	2	1	0.25	<0.125	0.5	0.5
15	>2	0.5	0.5	0.5	>2	0.125	1	2	1	0.25	<0.125	0.5	0.5

Table 6: continued

Isolate no.	B663 (µg/ml)	B4103 (µg/ml)	B4112 (µg/ml)	B4119 (µg/ml)	B4125 (µg/ml)	B4126 (µg/ml)	B4127 (µg/ml)	B4128 (µg/ml)	B4158 (µg/ml)	B4163 (µg/ml)	B4169 (µg/ml)	B4180 (µg/ml)	B4322 (µg/ml)
16	>2	<0.125	0.5	0.5	>2	2	1	2	>2	1	0.5	0.5	1
17	>2	0.5	0.5	1	>2	0.25	1	2	1	0.25	0.5	0.5	1
18	>2	0.5	0.5	>0.125	>2	0.25	1	1	1	0.5	1	0.5	1
19	>2	0.25	0.5	0.5	<0.125	0.5	1	2	1	0.25	<0.125	0.5	2
20	>2	0.5	0.5	0.5	2	2	1	2	1	0.5	0.5	0.5	0.5
21	>2	0.5	0.5	0.5	>2	2	1	2	1	1	1	0.5	0.5
22	>2	0.5	0.5	0.5	2	1	1	1	1	1	1	0.5	0.5
23	>2	0.5	0.5	0.5	2	1	2	2	1	0.5	0.5	0.5	0.5
24	>2	0.5	0.5	0.5	>2	1	2	2	2	1	1	0.5	1

EXAMPLE 5

The procedure of Example 2 was followed using -

(a) *Staphylococcus aureus* strains that were resistant to methicillin,

(b) *Enterococcus* strains that were resistant to high levels of penicillin, ampicillin and gentamicin and

(c) *Streptococcus pneumoniae* strains that were resistant to penicillin, cefotaxime, tetracycline, erythromycin, clindamycin and rifampicin.

The tests were carried out in triplicate and the results given were averaged and are set out in Table 7 below.

Table 7:

Drugs tested	Percentage Susceptible Clinical Isolates							
	Methicillin-resistant *Staphylococcus aureus* n=24				*Enterococcus* species n=36		*Streptococcus pneumoniae* n=10	
	0.25 μg/ml	0.5 μg/ml	1 μg/ml	2 μg/ml	1 μg/ml	2 μg/ml	1 μg/ml	2 μg/ml
B663	8.33	20.83	25	33.33	0	0	50	100
B4103	91.66	100	100	100	0	72.2	90	90
B4119	37.5	91.66	100	100	0	41.66	30	100
B4121	29.16	45.83	87.5	100	0	0	20	90
B4125	20.83	95.83	100	100	66.6	100	100	100
B4126	41.66	87.5	95.83	100	0	80.55	10	90
B4163	8.33	16.66	62.50	95.83	0	0	30	70

Methicillin is a semi-synthetic ß-lactam antibiotic, resistant to enzymatic hydrolysis by ß-lactamase enzymes. Methicillin resistance rates among staphylococci range from about 10 to 50 % in hospitals throughout the United States, and rates are comparable in several other countries. Nosocomial methicillin-resistant staphylococci are usually resistant to several other antibiotics such as erythromycin, clindamycin, tetracycline, chloramphenicol and gentamicin. These strains should also be considered resistant to all ß-lactam antibiotics, regardless of *in vitro* susceptibility. The presence of the penicillin-binding protein 2a in methicillin-resistant staphylococci has been linked with therapeutic failure of ß-

lactam antibiotics of penicillin, cephalosporin and penem classes, indicating cross-resistance to these antibiotics *in vivo*.

As can be seen from the table, the drugs of choice for the enterococci are B4125 and B4126, with B4125 already inhibiting 66.6% of strains at a concentration of 1 μg/ml. The most active drugs against the methicillin-resistant staphylococci are B4119, B4126, B4125 and B4121, and at a concentration of 0.5 μg/ml, B4125 inhibited 95.8 % of all strains. These correspond very well to the results obtained for the pneumococci.

The susceptibilities of the strains used, and of a vancomycin-resistant *Enterococcus faecium* strain are set out in the following Table 8.

Table 8:

Drugs tested	Susceptibilities of ATCC strains and a vancomycin-resistant *Enterococcus faecium* strain							
	Staphylococcus aureus ATCC 29213		*Enterococcus faecalis* ATCC 29212		*Streptococcus pneumoniae* ATCC 49619		Vancomycin-resistant *Enterococcus faecium*	
	0.25 μg/ml	0.5 μg/ml	1 μg/ml	2 μg/ml	1 μg/ml	2 μg/ml	1 μg/ml	2 μg/ml
B663	R	R	R	R	R	S	R	R
B4103	S	S	R	S	S	S	R	S
B4119	S•	S	R•	R	R	S	R	S
B4121	R	R	R	R	R	S	R	R
B4125	S	S	R	S	S	S	S	S
B4126	S	S	R	S	S	S	S	S
B4163	S	S	R	R	R	S	R	R

S•: strain susceptible, i.e. growth is inhibited at this concentration.

R•: strain resistant, i.e. growth is not inhibited at this concentration.

EXAMPLE 6

This Example gives details of a comparative intracellular test using B4121, B4125, B4128 and B4169 against rifampicin and clofazimine (B663).

Methods used

Mononuclear leucocytes were isolated from venous blood obtained from normal, healthy volunteers by density gradient centrifugation. For each treatment 2 ml of a suspension of mononuclear leucocytes (containing 1×10^5 monocytes) in RPMI medium supplemented with 10 % autologous serum were incubated in glass tubes at 37 °C for 60 min, after which the non-adherent cells were removed by washing. The cultures were then incubated in 2 ml RPMI medium supplemented with 10 % autologous serum for 2 weeks, washed and infected with a homogenous suspension of *M. tuberculosis* H37Rv (ATCC 25618) (resulting in an infectivity index of not more than 2 bacilli per cell) for 45 min at 37 °C in a shaking waterbath, rewashed and exposed for 2 days in 2 ml of culture medium to the antimicrobial agents.

The intracellular bacteria were released by re-suspending the cells in diluting fluid (BACTEC) and dispersing the clumps with a tuberculin syringe. Vials containing Middlebrook 4h12 (BACTEC 12B) medium were inoculated with the bacterial suspensions and incubated at 37 °C for up to 7 days. The growth index was recorded daily until the vials containing the untreated control suspensions reached ≥600 cpm.

The results are shown in Figure 4 of the accompanying drawings. As can be seen B4121, B4169, B4125, and to a lesser extent B4128, possess intracellular activity at concentrations as low as 0.005 μg/ml and were far superior to B663 as well as to the classical anti-tuberculosis agent rifampicin.

EXAMPLE 7 (comparison example)

This example gives a comparison between the activities of prior art compounds B663, B3962 and B4070, and some of the most versatile and effective new compounds covered by the present invention, namely B4112, B4119, B4121, B4125, B4126, B4128, B4158 and B4169.

B3962 is a compound of general formula (II), in which R^1n, R^2 and R^4n are all hydrogen. B4070 is a compound of general formula (II), in which R^1n and R^4n are 4-methyl and R^2 is hydrogen.

Method used

The results shown in Table 9 are from the same experiment as described in Example 4.

Table 9: Activity of Clofazimine and its derivatives against isolates of *Staphylococcus aureus*

Isolate no.	B663 (μg/ml)	B3962 (μg/ml)	B4070 (μg/ml)	B4112 (μg/ml)	B4119 (μg/ml)	B4121 (μg/ml)	B4125 (μg/ml)	B4126 (μg/ml)	B4128 (μg/ml)	B4158 (μg/ml)	B4169 (μg/ml)
ATCC	>2	>2	>2	2	1	>2	1	0.25	>2	1	0.5
2	>2	>2	>2	2	0.5	>2	>2	>2	>2	>2	>2
2	>2	>2	2	1	1	>2	<0.125	0.25	>2	1	<0.125
3	>2	>2	2	2	1	>2	>2	>2	>2	>2	>2
4	>2	>2	2	2	0.5	>2	>2	>2	>2	>2	>2
5	>2	>2	2	1	2	>2	>2	>2	>2	>2	2
6	>2	>2	2	1	2	>2	<0.125	0.25	>2	1	0.5
7	>2	>2	2	1	1	>2	1	>2	2	1	0.25
8	>2	>2	2	1	2	>2	1	>2	2	2	0.25
9	>2	>2	>2	1	2	>2	>2	>2	>2	>2	0.25
10	>2	>2	2	2	1	>2	<0.125	>2	2	1	0.25
11	>2	>2	2	0.5	1	>2	<0.125	>2	2	1	<0.125
12	>2	>2	2	1	0.5	>2	>2	>2	2	>2	>2
13	>2	>2	2	0.5	0.5	1	>2	<0.125	2	1	<0.125
14	>2	>2	2	0.5	0.5	>2	<0.125	<0.125	2	1	<0.125
15	>2	>2	2	0.5	0.5	>2	>2	<0.125	2	1	<0.125
16	>2	>2	2	0.5	0.5	>2	>2	2	2	>2	0.5
17	>2	>2	2	0.5	1	0.5	>2	0.25	2	1	0.5
18	>2	>2	2	0.5	1	1	>2	0.25	2	1	1

Table 9: continued

Isolate no.	B663 (μg/ml)	B3962 (μg/ml)	B4070 (μg/ml)	B4112 (μg/ml)	B4119 (μg/ml)	B4121 (μg/ml)	B4125 (μg/ml)	B4126 (μg/ml)	B4128 (μg/ml)	B4158 (μg/ml)	B4169 (μg/ml)
19	>2	>2	<0.125	0.5	0.5	>2	<0.125	0.5	2	1	<0.125
20	>2	>2	1	0.5	0.5	0.5	2	2	2	1	0.5
21	>2	>2	2	0.5	0.5	>2	>2	2	2	1	1
22	>2	>2	2	0.5	0.5	>2	2	1	1	1	1
23	>2	>2	2	0.5	0.5	>2	2	1	2	1	0.5
24	>2	>2	2	0.5	0.5	>2	>2	1	2	2	1

Example 8

This example illustrates the excellent anti-malarial activity of B4119 and B4125 against a laboratory strain of *P.falciparum*.

A laboratory strain of *P.falciparum* (RB-1 obtained from Dr B L Sharp, National Malarial Research Programme, MRC, Durban, South Africa) was maintained. Malarial cultures of haematocrit 5% and initial parasitemeia 2% were used. The malarial infected erythrocytes (ring stage) were incubated in microtitre plates with serial dilution of B4119 and B4125 (0.125 - 2 μg/ml) for 48 hours and processed for analysis on a flow cytometer.

The effects of B4119 and B4125 on the growth of *P.falciparum* were as follows:

Table 10:

Concentration	Percentage inhibition B4119	Percentage inhibition B4125
0.125	49	30
0.250	73	53
0.500	95	85
1.000	100	93
2.000	100	100

The results, as well as for comparison compounds B4112 and 4158, are illustrated in Figure 5 of the accompanying drawings.

The previously best anti-malarial compounds found were the known compounds (see EPO 729 737) B4158 and B4112, both of which were superior to clofazimine. These two compounds are included for comparison purposes. The higher activity of B4119 and B4125 at lower doses is clearly demonstrated.

B4158 is a compound of formula (I) where R^1 and R^4 are 4-isopropyl, R_2 is hydrogen, R^3 is 4'-TMP and n is 1. B4112 is a compound of formula (I) in which R^1 are R^4 are 3-chloro, R^2 is hydrogen, R^3 is 4'-TMP and n is 1.

Example 9

This example illustrates the MDR activity of B4119 and B4125.

The effects were examined over a seven (7) day exposure to different concentrations of B4119 and B4125 on the sensitivity of a doxorubicin resistant leukaemia cell line (K562/MMB) and a lung carcinoma cell line (H69/LX4) to doxorubicin (dox) and vinblastine (VB) using the MTT assay.

The cells were seeded at 1 x 10^4 cells per well in 96 well microtitre plates in a volume of 200 μl of RPMI 1640 medium containing 10 % fetal calf serum and incubated with doxorubicin (12.5 ng/ml) or vinblastine (3 ng/ml and 25 ng/ml). The amounts of MDR modulators used were 0.03, 0.06, 0.125, 0.5, 1.0 and 2.0 μg/ml.

After incubation at 37 °C for 7 days, 20 μl MTT (i.e. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) at 5 mg/ml was added to each well and the plates incubated for a further 4 hours. The cells were washed with phosphate buffered saline and the intracellular formazan crystals solubilized with dimethylsulphoxide, and the absorbency measured spectrophometically at a test wavelength of 540 nm and a reference wavelength of 620 nm. The results obtained are shown in Table 11. In this table the IC 50 (μg/ml)* of B4119 and B4125 are expressed as the mean drug concentration (μg/ml) causing 50% cell killing in 2-4 experiments. The concentrations of doxorubicin and vinblastine (present with the riminophenazine) possessed minimal cytotoxic activity (less than 10%).

Table 11:

Compound	IC50* (μg/ml)					
	K562/MMB			H69/LX4		
	none	+dox (12ng/ml)	+VB (3ng/ml)	none	+dox (12ng/ml)	+VB (25ng/ml)
B4125	0.311	0.188	0.026	0.785	0.521	0.399
B4119	0.276	0.147	0.189	0.620	0.600	0.109

Example 10

This example illustrates the *in vitro* results of the anti-tumour activity of B4119 and B4125.

The sensitivity of MDR cell lines derived from human colon, oesophagus and prostate for each of B4119 and B4125 was determined and compared with cis-platin.

Cell Lines:

The following cell lines were used:

i. Human colorectal carcinoma:
CaCo2 (ATC HTB 37); Colo 320 DM (ATCC CCL 220);
HT-29 (ATCC HTB 38);

ii. Human prostate carcinoma, metastasis to brain:
DU-145 (ATCC HTB 81);

iii. Human oesophagus carcinoma:
$WHCO_3$; $WHCO_6$.

The human oesophagus carcinoma cell lines are available from the Department of Zoology and Histo-Pathology, University of the Witwatersrand.

Cytotoxicity assays:

The effects of cis-platin and the riminophenazines (at 0.03 - 2 μg/mℓ) on the carcinomatous cell lines were examined after a 3-4 day exposure time using the MTT assay for the only non-adherent cell line (Colo 320 DM) and a crystal violet staining method for the remainder of the cell lines.

Results:

The sensitivities (expressed as IC50) of the cell lines to B4119 and B4125, are shown in **Table 12**. The mean IC50's of B4119 and B4125 for all the cell lines tested ranged between 0.0252 and 1.523 μg/ml and most of them compared favourably with the mean IC50 of cis-platin.

Table 12

Table 12: **Chemosensitivity of human cancer cell lines**

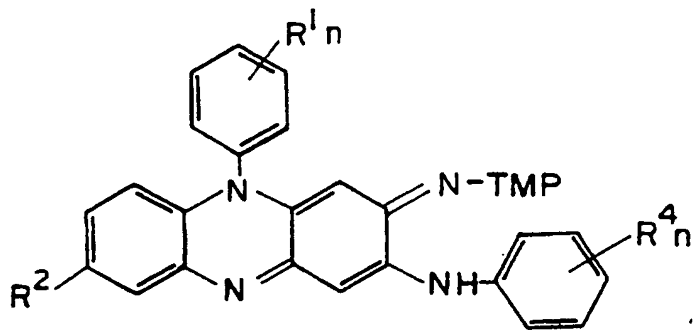
Compound	IC50* (μg/ml) for cell lines						
	Colo	HT-29	$CaCo_2$	DU-145	$WCHO_3$	$WCHO_6$	Mean IC50
B4119	**0.384**	0.427	0.421	0.317	nd	nd	0.387
B4125	**0.375**	0.149	0.128	0.309	0.283	0.269	0.252

* Data from 2-4 experiments are expressed as the mean drug concentration (μg/ml) causing 50 % cell kill.

nd means not determined.

CLAIMS:

1. A substance or composition for use in a method of treatment of a gram-positive bacterial infection of the human or animal body by the administration of said substance or composition to the human or animal body, said substance or composition comprising a compound of formula (II):



(II)

in which R^1 and R^4 are the same and each is a halogen atom, a haloalkyl radical or an isopropyl radical, n is 1, 2 or 3 and represents the number of R^1 substituents and of R^4 substituents, R^2 is hydrogen, halogen or haloalkyl, and TMP is a tetramethyl piperidyl radical, with the provisos that (a) when n is 2, R^1n and R^4n are not 3,4-dichloro- and (b) when n is 1, R^1 and R^4 are not 4-trifluoromethyl-.

2. A substance or composition according to claim 1, wherein the bacterial infection is by any one or more of the species selected from *Mycobacterium aurum, Mycobacterium tuberculosis, Mycobacterium chelonae, Mycobacterium abscessus, Mycobacterium fortuitus, Streptococcus pneumoniae, Enterococcus* sp. and *Staphylococcus aureus.*

3. A substance or composition according to claims 1 or 2, wherein R^1 and R^4 are selected from the group consisting of chlorine, bromine, fluorine, isopropyl, 2-trifluoromethyl and 3-trifluoromethyl, and R^2 is hydrogen or chlorine.

4. A substance or composition according to claim 3, wherein n is 1 and the substituents R^1n and R^4n are selected from the group consisting of 2-chloro-; 3-chloro-; 3-trifluoromethyl-; 3-bromo-; 3-fluoro- and 4-isopropyl-; or wherein n is greater than 1 and the substituents R^1n and R^4n are selected from the group consisting of 2-chloro-4-fluoro-; 3,5-dichloro-; 2,4-dichloro-; 3-trifluoromethyl- 4-chloro- and 3,4, 5-trifluoromethyl-.

5. A substance or composition according to claim 3, wherein the compound is selected from the group consisting of:

(a) N,5-*bis*(3-chlorophenyl)-3-5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(b) N,5-*bis*(3-chloro-4-fluorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(c) N,5-*bis*(3,5-dichlorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(d) N,5-*bis*(2-chlorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(e) N,5-*bis*(3-trifluoromethylphenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(f) N,5-*bis*(3-trifluoromethylphenyl)-8-chloro-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(g) N,5-*bis*(2,4-dichlorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(h) N,5-*bis*(4-isopropylphenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

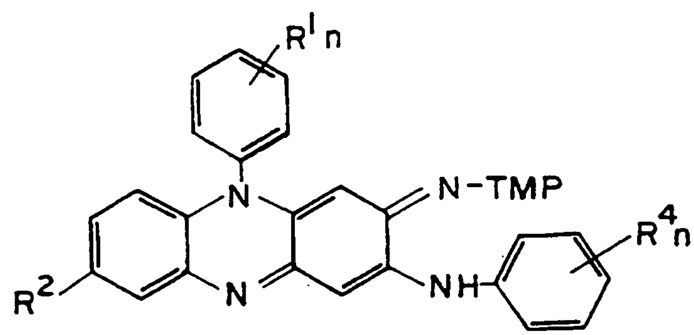
(i) N,5-*bis*(3-trifluoromethyl-4-chlorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(j) N,5-*bis*(3,4,5-trichloromethyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(k) N,5-*bis*(3-bromophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine; and

(l) N,5-*bis*(3-fluorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine.

6. The use, in the manufacture of a substance or composition for administration to persons or animals suffering from a bacterial infection by gram-positive bacteria to treat the infection, of a compound of the formula (II):



(II)

in which R^1 and R^4 are the same and each is a halogen atom, a haloalkyl radical or an isopropyl radical, n is 1, 2 or 3 and represents the number of R^1 substituents and of R^4 substituents, R^2 is hydrogen, halogen or haloalkyl, and TMP is a tetramethyl piperidyl radical, with the provisos that

(a) when n is 2, R^1n and R^4n are not 3,4-dichloro-; and

(b) when n is 1, R^1 and R^4 are not 4-trifluoromethyl.

7. The use according to claim 6, wherein the bacterial infection is by any one or more of the species selected from *Mycobacterium aurum*, *Mycobacterium tuberculosis*, *Mycobacterium chelonae*, *Mycobacterium abscessus*, *Mycobacterium fortuitus*, *Streptococcus pneumoniae*, *Enterococcus* sp. and *Staphylococcus aureus*.

8. The use according to claims 6 or 7, wherein R^1 and R^4 are selected from the group consisting of chlorine, bromine, fluorine, isopropyl, 2-trifluoromethyl and 3-trifluoromethyl, and R^2 is hydrogen or chlorine.

9. The use according to claim 8, wherein n is 1 and the substituents R^1n and R^4n are selected from the group consisting of 2-chloro-; 3-chloro-; 3-trifluoromethyl-; 3-bromo-; 3-fluoro- and 4-isopropyl-; or wherein n is greater than 1 and the substituents R^1n and R^4n are selected from the group consisting of 2-chloro-4-fluoro; 3,5-dichloro-; 2,4-dichloro-; 3-trifluoromethyl- 4-chloro- and 3,4, 5-trifluoromethyl-.

10. The use according to claim 8, wherein the compound is selected from the group consisting of:

(a) N,5-*bis*(3-chlorophenyl)-3-5-dihydro-3-[(2′,2′,6′,6′-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(b) N,5-*bis*(3-chloro-4-fluorophenyl)-3,5-dihydro-3-[(2′,2′,6′,6′-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(c) N,5-*bis*(3,5-dichlorophenyl)-3,5-dihydro-3-[(2′,2′,6′,6′-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(d) N,5-*bis*(2-chlorophenyl)-3,5-dihydro-3-[(2′,2′,6′,6′-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(e) N,5-*bis*(3-trifluoromethylphenyl)-3,5-dihydro-3-[(2′,2′,6′,6′-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(f) N,5-*bis*(3-trifluoromethylphenyl)-8-chloro-3,5-dihydro-3-[(2′,2′,6′,6′-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(g) N,5-*bis*(2,4-dichlorophenyl)-3,5-dihydro-3-[(2′,2′,6′,6′-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(h) N,5-*bis*(4-isopropylphenyl)-3,5-dihydro-3-[(2′,2′,6′,6′-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

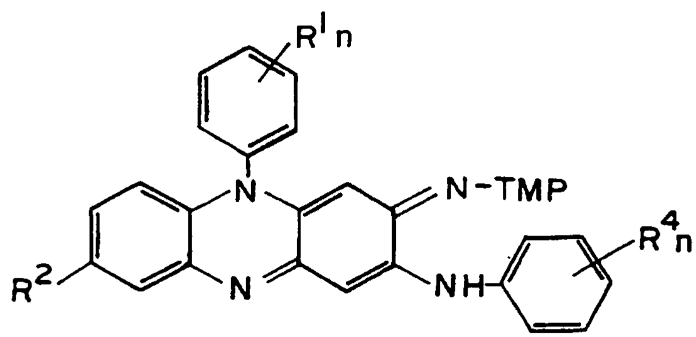
(i) N,5-*bis*(3-trifluoromethyl-4-chlorophenyl)-3,5-dihydro-3-[(2′,2′,6′,6′-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(j) N,5-*bis*(3,4,5-trichloromethyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(k) N,5-*bis*(3-bromophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine; and

(l) N,5-*bis*(3-fluorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine.

11. A method of treating a human or animal patient suffering from a bacterial infection by gram-positive bacteria, which comprises administering to the human or animal patient an effective amount of a compound of the formula (II):



(II)

in which R^1 and R^4 are the same and each is a halogen atom, a haloalkyl radical or an isopropyl radical, n is 1, 2 or 3 and represents the number of R^1 substituents and of R^4 substituents, R^2 is hydrogen, halogen or haloalkyl, and TMP is a tetramethyl piperidyl radical, with the provisos that

(a) when n is 2, R^1n and R^4n are not 3,4-dichloro-; and

(b) when n is 1, R^1 and R^4 are not 4-trifluoromethyl.

12. A method according to claim 1, wherein the bacterial infection is by any one or more of the species selected from *Mycobacterium aurum, Mycobacterium tuberculosis, Mycobacterium chelonae, Mycobacterium abscessus, Mycobacterium fortuitus, Streptococcus pneumoniae, Enterococcus* sp. and *Staphylococcus aureus.*

13. A method according to claims 11 or 12, wherein R^1 and R^4 are selected from the group consisting of chlorine, bromine, fluorine, isopropyl, 2-trifluoromethyl and 3-trifluoromethyl, and R^2 is hydrogen or chlorine.

14. A method according to claim 13, wherein n is 1 and the substituents R^1n and R^4n are selected from the group consisting of 2-chloro-; 3-chloro-; 3-trifluoromethyl-; 3-bromo-; 3-fluoro- and 4-isopropyl-; or wherein n is greater than 1 and the substituents R^1n and R^4n are selected from the group consisting of 2-chloro-4-fluoro-; 3,5-dichloro-; 2,4-dichloro-; 3-trifluoromethyl-4-chloro and 3,4 5-trifluoromethyl-.

15. A method according to claim 13, wherein the compound is selected from the group consisting of:

(a) N,5-*bis*(3-chlorophenyl)-3-5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(b) N,5-*bis*(3-chloro-4-fluorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(c) N,5-*bis*(3,5-dichlorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(d) N,5-*bis*(2-chlorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(e) N,5-*bis*(3-trifluoromethylphenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(f) N,5-*bis*(3-trifluoromethylphenyl)-8-chloro-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(g) N,5-*bis*(2,4-dichlorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(h) N,5-*bis*(4-isopropylphenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(i) N,5-*bis*(3-trifluoromethyl-4-chlorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(j) N,5-*bis*(3,4,5-trichloromethyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine;

(k) N,5-*bis*(3-bromophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine; and

(l) N,5-*bis*(3-fluorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine.

16. A pharmaceutically active compound, said compound being N,5-*bis*(3-chloro-4-fluorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine.

17. A pharmaceutically active compound, said compound being N,5-*bis*(2-chlorophenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine.

18. The use, in the treatment of a parasitic infection, of a compound according to claim 16 or 17.

19. The use, in the manufacture of a pharmaceutical composition for administration to a person or animal suffering from a parasitic infection, of a compound according to claims 16 or 17.

20. A method for the prophylactic and/or therapeutic treatment of a parasitic infection of the human or animal body, which comprises administering to said human or animal body an effective amount of a compound according to claims 16 or 17.

21. The use as claimed in claim 18 or claim 19, or a method as claimed in claim 20, wherein the parasitic infection is malaria.

22. A pharmaceutical composition comprising a compound according to claim 16 or claim 17 and a pharmaceutically acceptable carrier.

23. A method for the preparation of a compound according to claim 16 or claim 17, which comprises reacting a corresponding compound, in which the imino group in the 3- position is unsubstituted, with 4-amino-(2,2,6,6-tetramethyl) piperidine.

24. A substance or composition for use in a method of treatment of a bacterial infection of the human or animal body caused by bacteria of the genus *Streptococcus, Enterococcus* or *Staphylococcus*, said method of treatment comprising administering an effective amount of said substance or composition to the human or animal body, said substance or composition comprising N,5-*bis*(4-trifluoromethylphenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine.

25. The use, in the manufacture of a substance or composition for administration to persons or animals suffering from a bacterial infection by bacteria of the genus *Streptococcus, Enterococcus* or *Staphylococcus* to treat the infection, of the compound N,5-*bis*(4-trifluoromethylphenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine.

26. A method of treating a human or animal patient suffering from a bacterial infection by bacteria of the genus *Streptococcus, Enterococcus* or *Staphylococcus* which comprises administering to the human or animal patient an effective amount of N,5-*bis*(4-trifluoromethylphenyl)-3,5-dihydro-3-[(2',2',6',6'-tetramethyl-4-piperidyl)-imino]-2-phenazinamine.

GROWTH OF M. AURUM

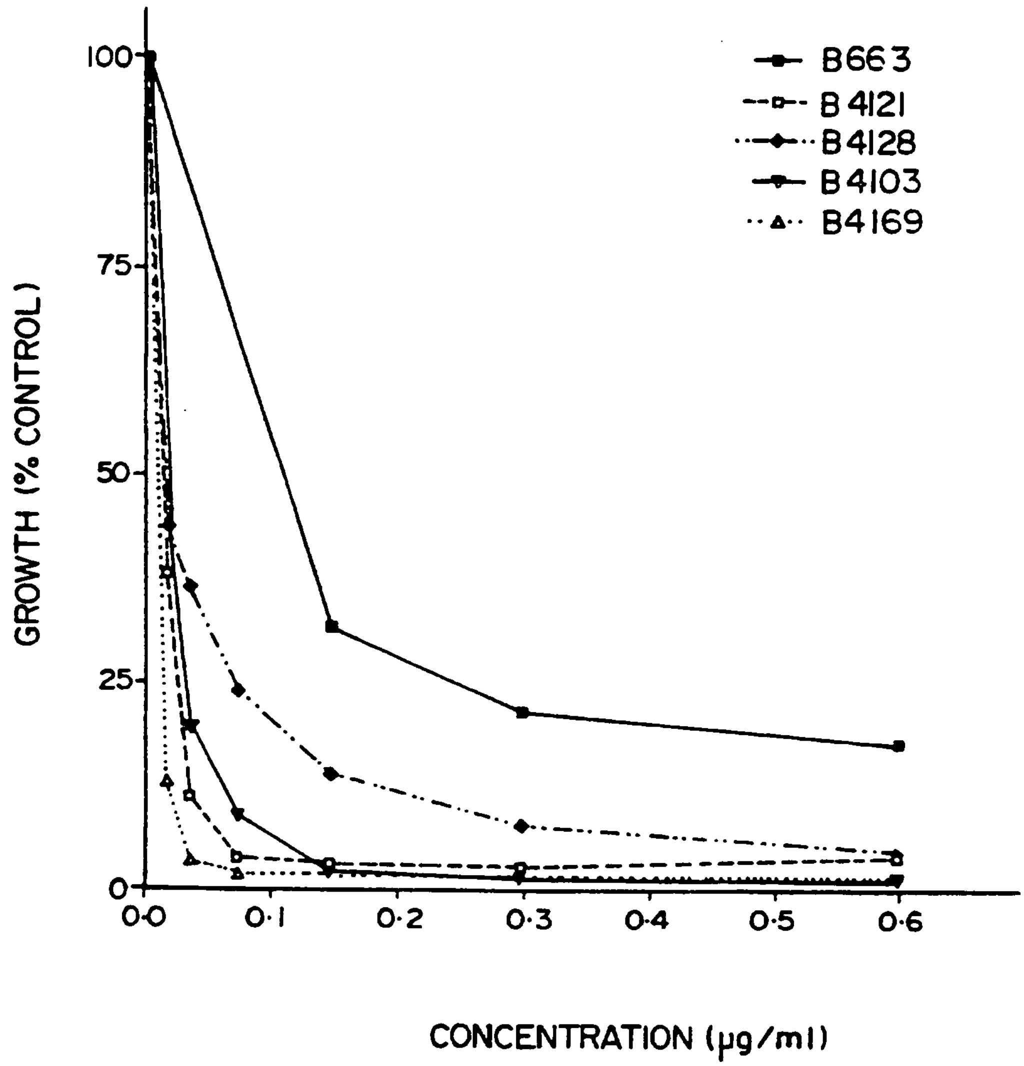


FIG 1

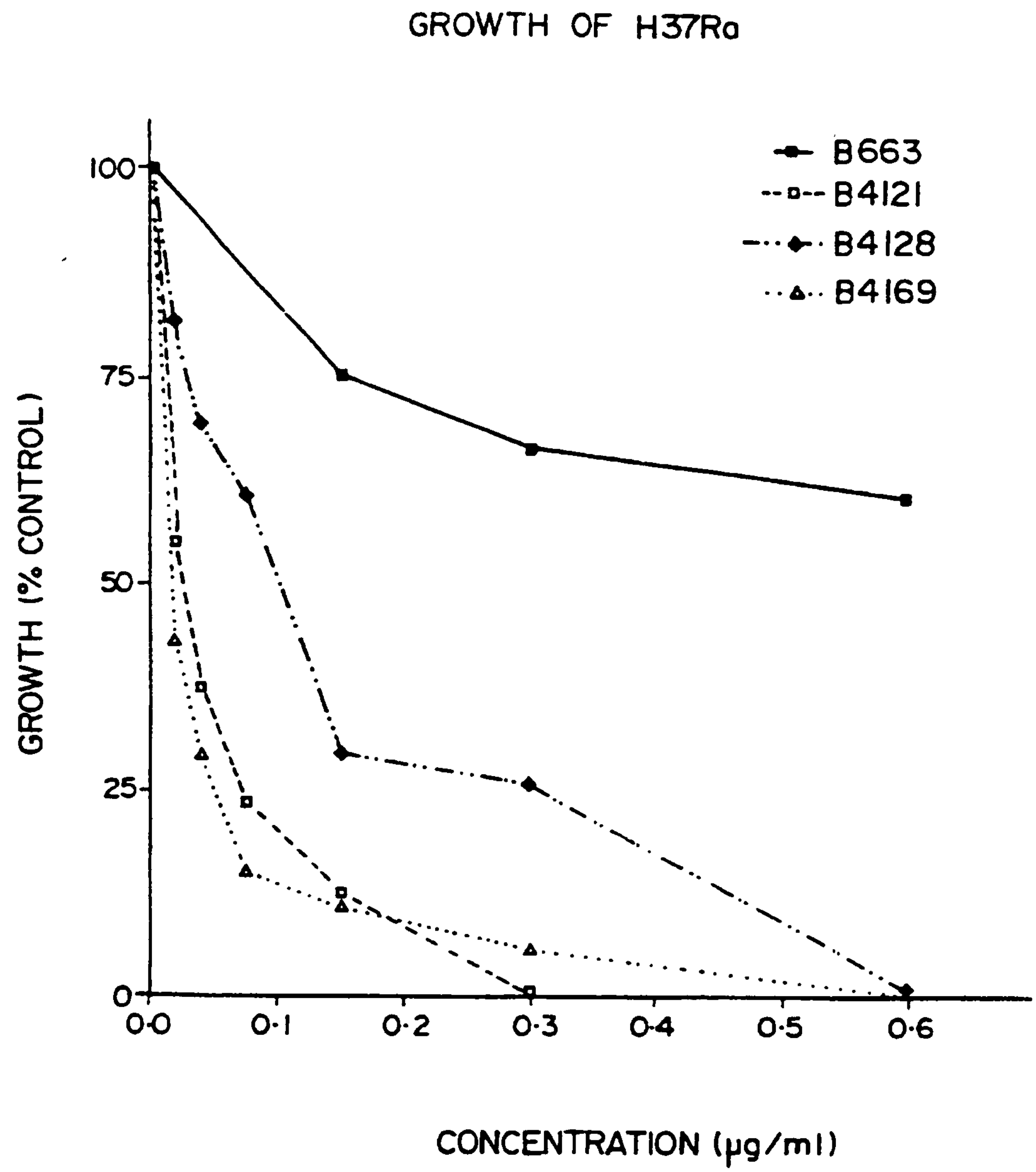

GROWTH OF H37Ra
B663
B4121
B4128
B4169
GROWTH (% CONTROL)
100
75
50
25
0
0·0
0·1
0·2
0·3
0·4
0·5
0·6
CONCENTRATION (μg/ml)

FIG 2

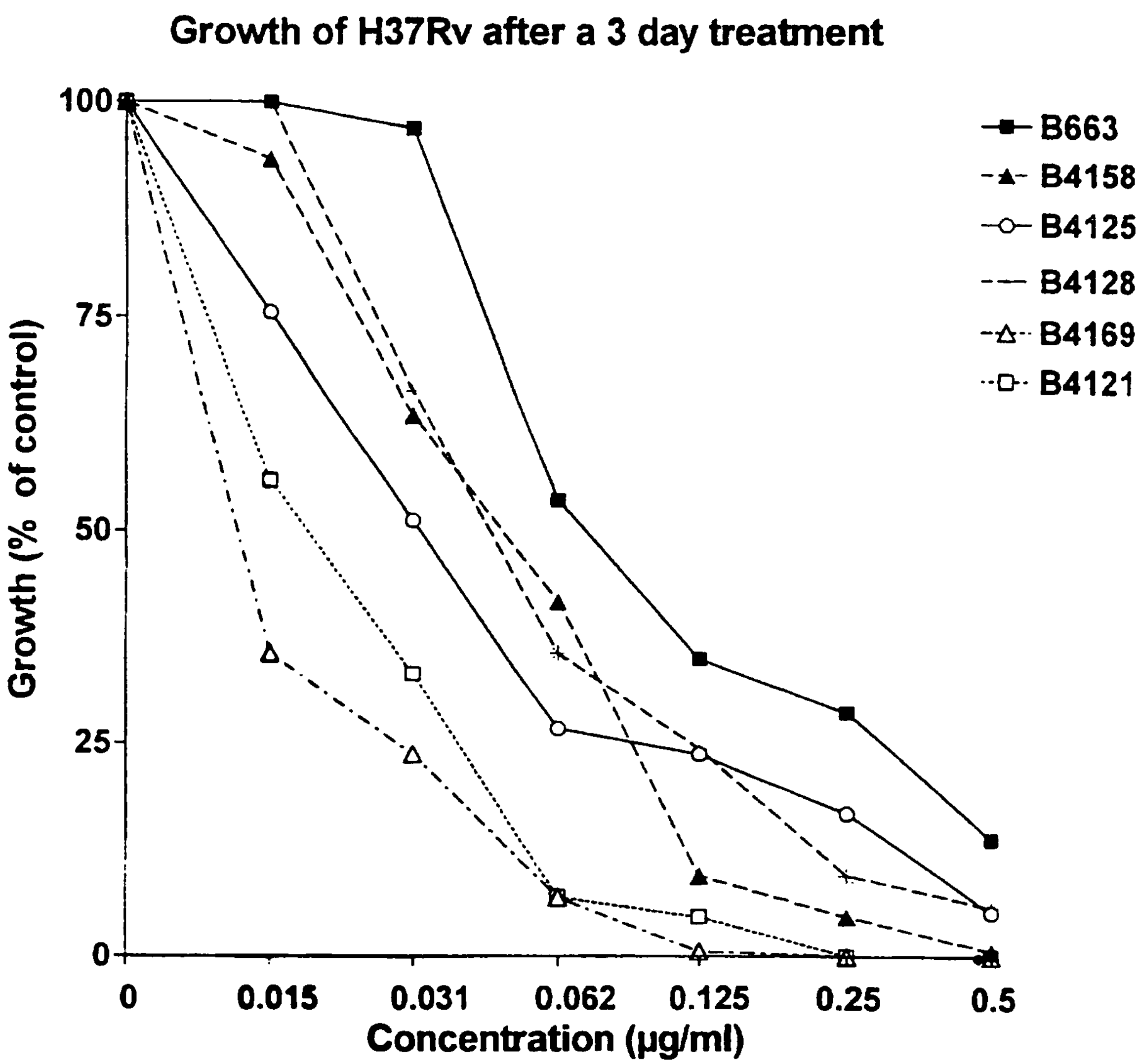

Growth of H37Rv after a 3 day treatment
Growth (% of control)
100
75
50
25
0
0
0.015
0.031
0.062
0.125
0.25
0.5
Concentration (µg/ml)
B663
B4158
B4125
B4128
B4169
B4121

FIG 3

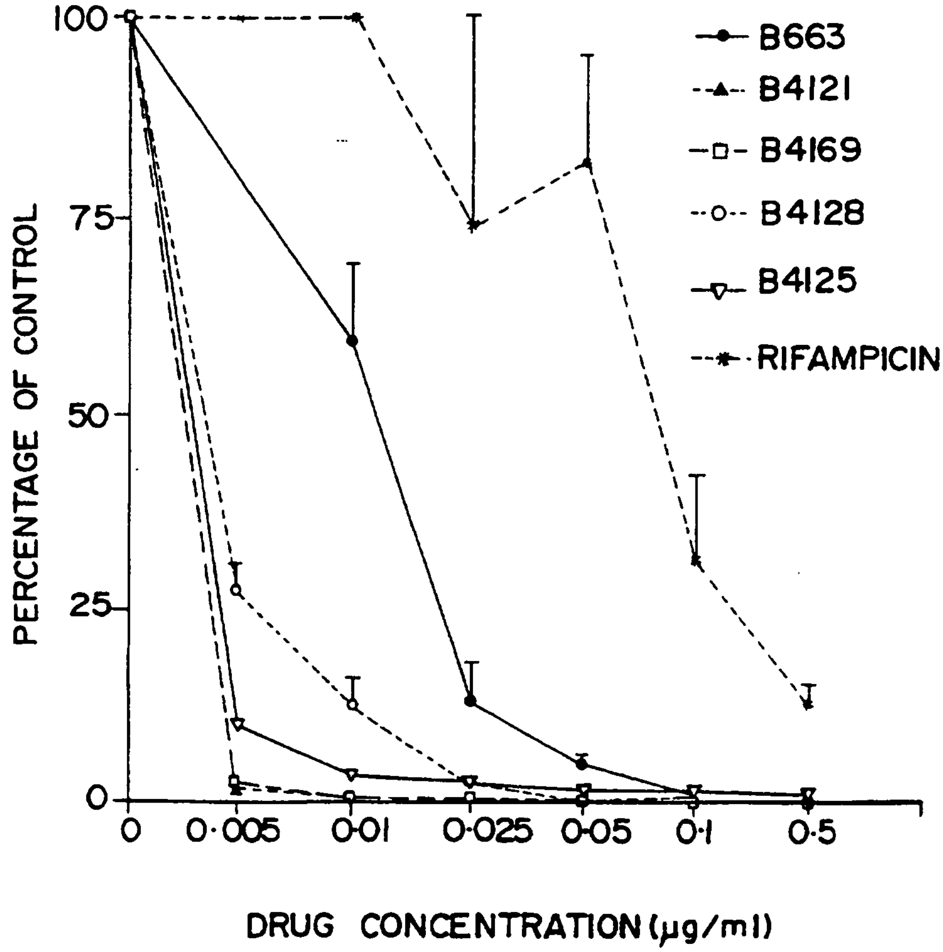

100
75
50
25
0
PERCENTAGE OF CONTROL
0
0·005
0·01
0·025
0·05
0·1
0·5
DRUG CONCENTRATION (µg/ml)
B663
B4121
B4169
B4128
B4125
RIFAMPICIN

FIG 4

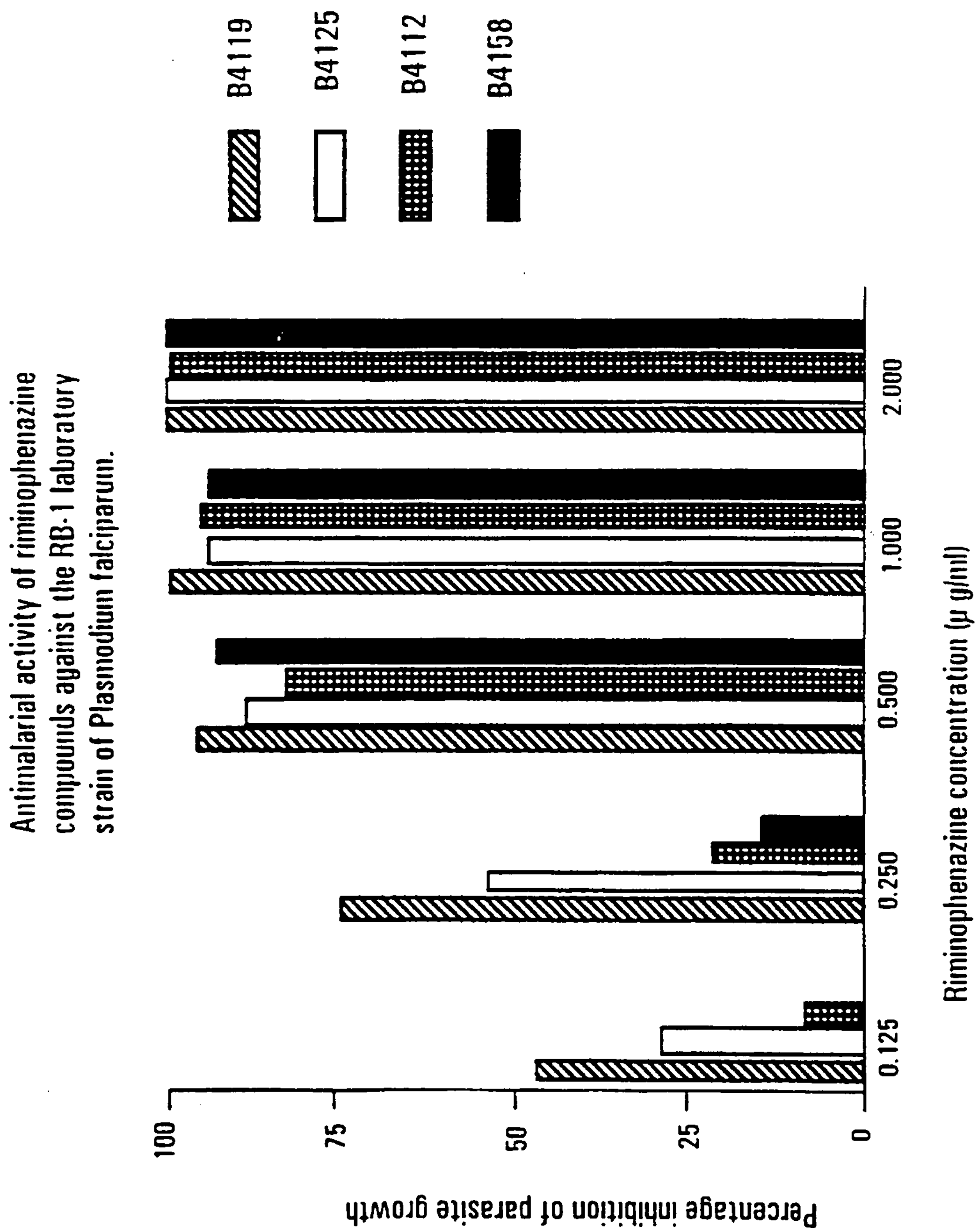

Antimalarial activity of riminophenazine compounds against the RB-1 laboratory strain of Plasmodium falciparum.
B4119
B4125
B4112
B4158
Percentage inhibition of parasite growth
100
75
50
25
0
0.125
0.250
0.500
1.000
2.000
Riminophenazine concentration (µ g/ml)

FIG 5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/01395

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/495 C07D241/46 C07D401/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R.M. VAN LANDINGHAM ET AL.: "Activity of phenazine analogs against Mycobacterium leprae infections in mice." INT. J. LEPRA OTHER MYCOBACT. DIS., vol. 61, no. 3, 1993, pages 406-414, XP002040580 see the whole document ---	1,3,6,8, 11,13
X	S.G. FRANZBLAU ET AL.: "Structure-activity relationships of tetramethylpiperidine-substituted phenazines against Mycobacterium leprae in vitro." ANTIMICROB. AGENTS CHEMOTHER., vol. 33, no. 11, 1989, pages 2004-2005, XP002040581 see the whole document ---	1,3,6,8, 11,13

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 1997	26.09.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Klaver, T

INTERNATIONAL SEARCH REPORT

Inte. .nal Application No
PCT/GB 97/01395

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EP 0 729 757 A (ADCOCK INGRAM LTD) 4 September 1996 cited in the application see table 1 ---	1,3-5
A	EP 0 676 201 A (ADCOCK INGRAM LTD) 11 October 1995 cited in the application -----	

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/01395

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 11-15, 20, 26
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/01395

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 729757 A	04-09-96	AU 4219096 A	08-08-96
		CA 2168139 A	01-08-96
		JP 8231401 A	10-09-96
EP 676201 A	11-10-95	AU 1498295 A	12-10-95
		CA 2144783 A	06-10-95
		JP 7277982 A	24-10-95